

## **Markers of Decompression Stress of Mass Stranded/Live Caught and Released vs. Single Stranded Marine Mammals**

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### **LONG-TERM GOALS**

Our goal is to develop a biomarker of decompression stress in cetaceans. After undertaking a baseline experiment in Steller sea lions, this project will compare and contrast health, body condition, and presence of bubbles with markers for decompression stress in mass stranded cetaceans, and compare these indices with single stranded individuals. Single stranded animals are often free of bubbles while bubbles are common in mass stranded animals. We will also obtain baseline samples from captive and wild caught and released animals as further controls.

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## OBJECTIVES

*Given the above observations and background we are testing the following hypotheses:*

1. Microparticle (MP) levels correlate with increasing decompression stress and the presence of post-dive inert gas bubbles in voluntary diving Steller sea lions.
2. Beach stranding restrains supersaturated divers from recompressing with resultant gas emboli to a greater degree for mass than single stranders. We will therefore compare health status, MP levels and the presence or absence of bubbles in these two groups.
3. Captive animals and animals that remain in shallow water such as those restrained briefly for health assessment in Sarasota Bay (n=10-15/year), will show different degrees of gas supersaturation and hence MP levels than stranded animals that have come in from deeper waters.

### *Specific Aims:*

**Aim 1:** Calibrate the relationship between decompression stress and MP levels.

**Aim 2:** Sample and analyze single and mass stranded dolphins for MP levels and associated data.

**Aim 3:** Sample and analyze MP and associated data from live-restrained dolphins.

## APPROACH

Aim 1. Working with Andrew Trites, and David Rosen, University of British Columbia, and Martin Haulena, Troy Neale and Nigel Waller, Vancouver Aquarium, the Open Water (OW) Research Laboratory houses 4 adult female Steller sea lions. These animals can voluntarily dive in a fjord to feed from fish delivery tubes placed at a pre set depth. We work with these animals to verify that MP levels correlate with decompression stress (number of dives, duration and depth) in marine mammals. This has been done by taking blood samples before and after a diving bout (8-12 dives) to depths up to 50 m. The MP samples are analyzed by Stephen Thom at the University of Pennsylvania. In addition, the metabolic cost, (using respirometry), and environmental variables have been measured during each dive series.

We also use B-mode ultrasound in collaboration with Dr Sophie Dennison, a board certified Veterinary Radiologist, to determine whether bubbles are present before and after the dive bout and correlate the presence of bubbles with the measured decompression stress and MP levels. These data will allow us to determine if MP levels correlate with decompression stress in marine mammals when accounting for a number of covariates.

Aims 2 and 3. Working with Kathleen Moore at the International Fund for Animal Welfare Marine Mammal Rescue and Research group in Yarmouth Port, MA, and Randall Wells, Chicago Zoological Society at Sarasota, FL we will sample stranded and health-assessment restrained dolphins during beaching, transport or deck restraint for changes in MP levels, ultrasound and other related data. Principal Component Analysis (PCA, Jolliffe IT, 2002, Principal component analysis. Springer, Berlin Heidelberg New York) will be used to determine which stressors correlate with changes in MP levels. Our data will include MP levels from a diverse case series of live cetaceans and pinnipeds in various degrees of acute and/or chronic stress, such as control animals in a captive (sea lions in Vancouver), or wild situation (wild caught bottlenose dolphins), mass stranded or live caught and single stranded

animals for whatever stressor (acute or chronic disease, predation, or abandonment during suckling). All the available data including MP level, decompression stress as measured by presence of bubbles defined in Aim 1), environmental variables, standard measures of body condition using length, weight, girth and blubber thickness measured by ultrasound, along with health assessment parameters, to be acquired with a concurrent NOAA Prescott grant that has been awarded, will be subjected to PCA to tease out the common threads between perceived stressors and variation in MP levels.

## WORK COMPLETED

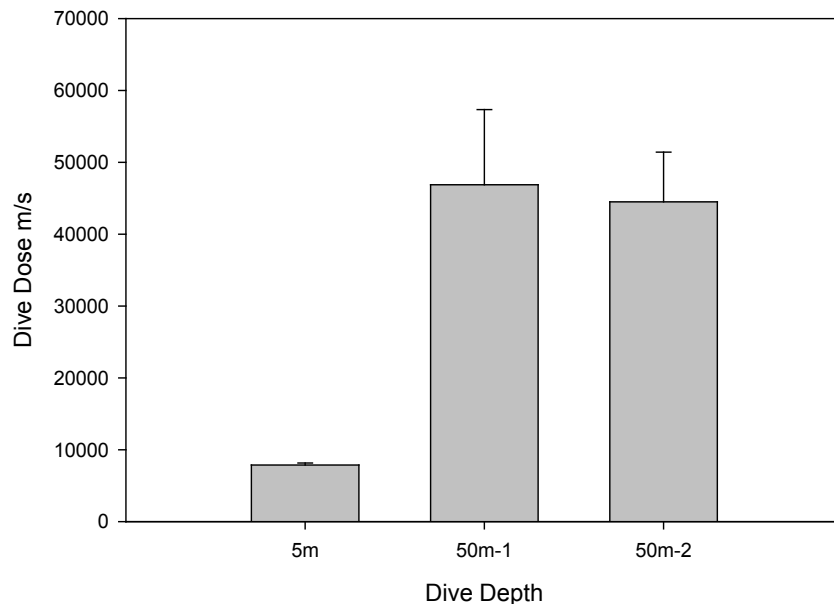
Aim 1. Two experiments have been undertaken at the OW facility in April and June 2012. Four Steller sea lions were sampled before, after, and 24 hours after dive bouts.

Aim 2. This will be initiated during the 2012/2013 stranding season.

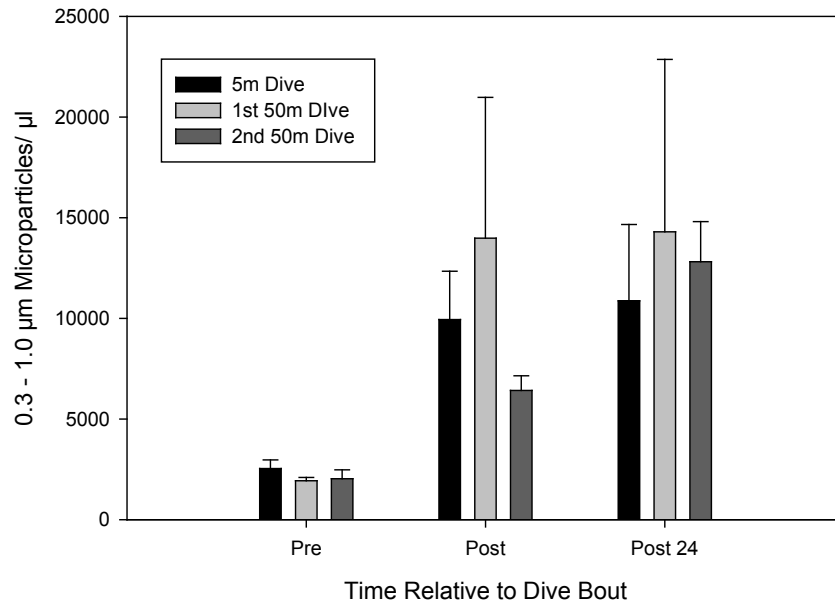
Aim 3. MP samples and ultrasound data were obtained from bottlenose dolphins undergoing health assessment in May and July 2012. Samples were taken at the beginning and end of the out of water period. Further samples will be collected in 2013.

## RESULTS

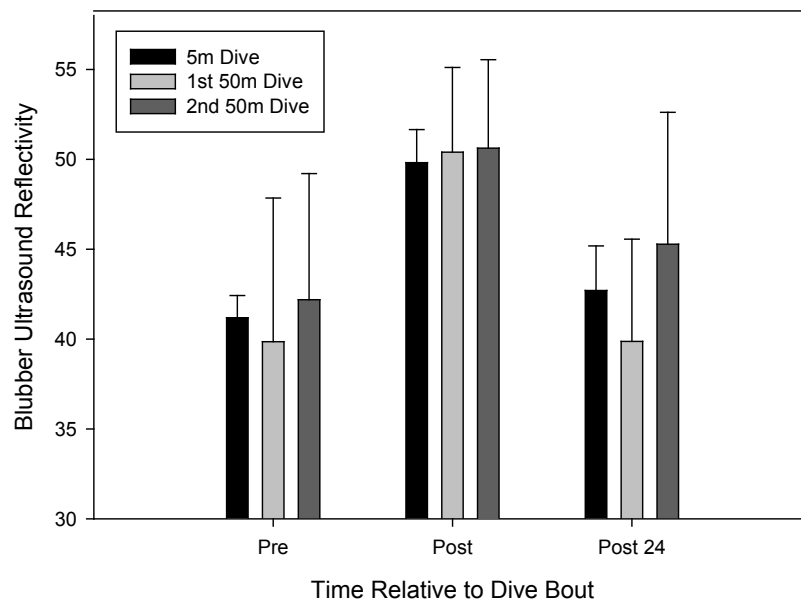
Aim 1. We have data from four female Steller sea lions trained to dive to selected depths at the UBC Open Water Facility in Vancouver.



***Figure 1 - An estimate of the 'dive dose' for 4 Steller sea lions which was calculated as the area under the depth time graph. This does not take into consideration the reduced gas exchange as the alveoli collapse with increasing depth.***



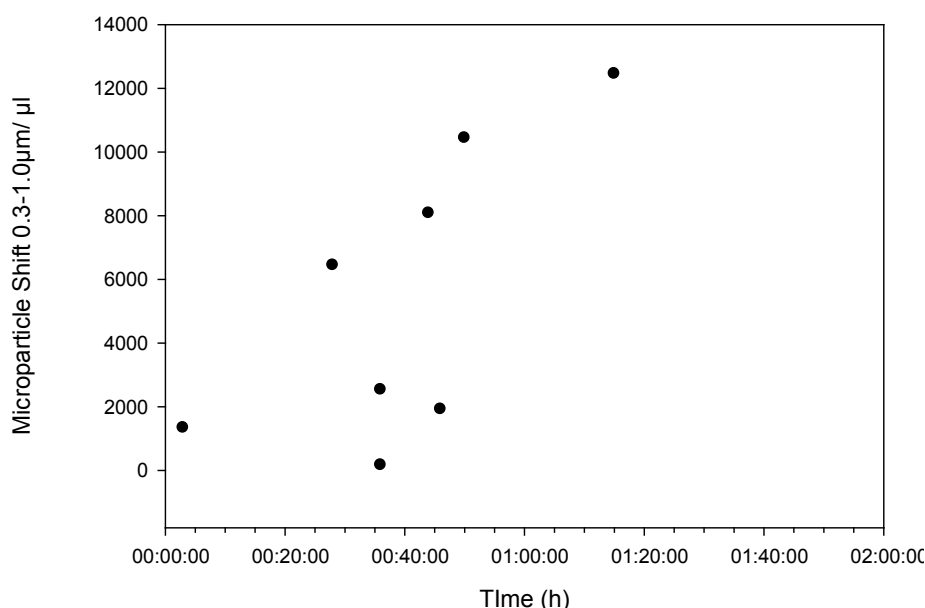
**Figure 2 - Microparticle counts for 4 Steller sea lions. Blood samples were taken immediately before (pre) a dive bout (to 5, or 50 m depth), an hour after the bout ended and 24 hours later. Note the consistent increase by 24h. The lack of difference between 5 and 50m may reflect the reduced gas exchange with increasing depth as alveolar collapse increases.**



**Figure 3 - Ultrasound reflectivity in the blubber for 4 Steller sea lions immediately before (pre) a dive bout (to 5, or 50 m depth), after the bout ended and 24 hours later. Note the consistent reversible increase post dive.**

**Table 1 - Estimated tissue end blood PN2 levels for different tissue compartment - means of 4 Steller sea lion dive bouts shown in Figures 1, 2 and 3. Pven is mixed venous or the overall animal saturation. Model (Fahlman et al. 2009) based on each animals dive trace and approximate body mass, with an assumed diving bradycardia of 50% with cardiac output scaled from a harbor seal. The lack of a difference between microparticle counts shown above, between 5m and 50m dives, would suggest that the slow tissue, the blubber, is the primary driving force in post dive decompression stress.**

Depth (m)	End dive PN2 (Atmospheres Absolute)				
	Heart	Muscle	Brain	Fat	Pven
5	1.084	1.057	1.081	0.770	1.063
50a	3.464	1.513	3.270	0.887	2.244
50b	3.518	1.509	3.370	0.892	2.274



**Figure 4 - Increase in microparticle count compared with time in Tursiops individuals that were held on deck during health assessment. Note the increasing trend seen with this data series obtained in July 2012 was the inverse of that observed in May 2012. Further data will be obtained to clarify this conundrum.**

Our plan going forward is to obtain surface swim Steller sea lion microparticle data to discriminate between exercise versus diving. We will serially sample stranded and boat restrained dolphins and explore options re getting serial samples from captive dolphins.

## IMPACT/APPLICATIONS

The results shown here are preliminary and await further analysis and a greater sample size. But we can at least conclude that we have shown a dive related increase in MPs and blubber ultrasound

reflectivity in Steller sea lions and changes in microparticles in bottlenose dolphins when held on the deck of a vessel. This is the first time, as far as we are aware, that microparticles, and in the context of diving, have been reported from any marine mammal. Microparticles are membrane vesicles shed from the surface of cells. They cause vascular neutrophil activation and injury. MP levels are positively related to the level of decompression stress in mice and humans (Thom et al. 2011).

These findings have potential implications for our understanding of how marine mammals manage gas solubility as it changes with depth (Hooker et al. 2012).

## **RELATED PROJECTS**

None

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